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Overweight, genetic susceptibility and LADA.

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Abbreviations:

ANDIS – All New Diabetics In Scania

AP – Attributable proportion due to interaction

EIRA – Epidemiological Investigation of Rheumatoid Arthritis

ESTRID – Epidemiological Study of Risk Factors for LADA and Type 2 diabetes

HUNT Study - Nord-Trøndelag Health Study

LADA – Latent Autoimmune Diabetes in Adults

Objectives

We investigated potential interactions between BMI and genotypes of human leukocyte antigen (HLA), *TCF7L2*-rs7903146 and *FTO*-rs9939609 in relation to the risk of latent autoimmune diabetes in adults (LADA) and type 2 diabetes.

Methods

We pooled data from two population-based studies; a Swedish study with incident cases of LADA (GADA positive, n=394) and type 2 diabetes (GADA negative, n=1,290) and matched non-diabetic controls (n=2,656) and a prospective Norwegian study, including incident cases

of LADA (n=131) and type 2 diabetes (n=1,901) and 886,120 person-years of follow-up. Analyses were adjusted for age, gender, physical activity and smoking. Interaction between overweight (BMI ≥25 kg/m^2) and HLA/ *TCF7L2*/ *FTO* high-risk genotypes was assessed by attributable proportion due to interaction (AP).

Results

The combination of overweight and high-risk genotypes of HLA, *TCF7L2* and *FTO* was associated with a RR_{pooled} of 7.59 (Confidence interval 5.27-10.93), 2.65 (1.97-3.56) and 2.21 (1.60-3.07), respectively, for LADA, compared to normal weight individuals with low/intermediate genetic risk. There was a significant interaction between overweight and HLA (AP=0.29, 0.10-0.47), *TCF7L2* (AP=0.31, 0.09-0.52) and *FTO* (AP=0.38, 0.15-0.61). The highest risk of LADA was seen in overweight individuals homozygous for the DR4 genotype (RR=26.76, 15.42-46.43, AP=0.58, 0.32-0.83 [Swedish data]). Overweight and *TCF7L2* also significantly interacted in relation to type 2 diabetes (AP=0.26, 0.19-0.33), however no interaction was observed with high-risk genotypes of HLA or *FTO*.

Conclusions

These results indicate that overweight interacts with HLA high-risk genotypes but also with genes associated with type 2 diabetes in the promotion of LADA.

In this large study we found that overweight significantly interacts with HLA high-risk genotypes, and with variants of TCF7L2 and FTO in relation to the risk of Latent Autoimmune Diabetes in Adults. .

Introduction

Overweight is by far the most influential environmental risk factor for type 2 diabetes (1). Accumulating evidence indicates that excessive weight may also promote autoimmune diabetes in both children (2) and adults (3). We recently showed that obesity increased the risk of latent autoimmune diabetes in adults (LADA) 3-6 fold and that the risk was particularly high in those with a combination of overweight and family history of diabetes (4). This suggests that the influence of overweight on the development of LADA may be modified by genetic susceptibility.

LADA is described as a hybrid form of diabetes with a pathogenesis that includes autoimmune destruction of the beta-cells as well as insulin resistance (5). Similar to type 1 diabetes, the genetic susceptibility to LADA is strongly linked to the human leucocyte antigen (HLA) gene complex, in particular DRB1 and DQB1 with the HLA-DRB1*04-DQB1*0302 and HLA-DRB1*0301-DQB1*0201 haplotypes conferring the highest risk (6-8). LADA has also been associated with variants increasing the risk of type 2 diabetes e.g. in the transcription factor 7-like 2 (*TCF7L2*) gene in some (9-11) but not in all (12,13) studies, including a recent GWAS (8), as well as with variants in the fat mass and obesity-associated *FTO* gene in the Norwegian HUNT Study (12).

In order to clarify the influence of overweight on the development of LADA we investigated the risk of LADA compared to type 2 diabetes in relation to interaction between overweight and a) HLA high-risk genotypes and b) risk variants in the *TCF7L2* and *FTO* loci. For this purpose, we used data from two large population-based Scandinavian studies with incident cases of LADA and type 2 diabetes.

Materials and Methods

The ESTRID Study

Study population: ESTRID (Epidemiological study of risk factors for LADA and type 2 diabetes; https://ki.se/imm/estrid) is a population-based case-control study described in detail previously (14). It is a sub-study of ANDIS (All New Diabetics in Scania; http://andis.ludc.med.lu.se), a large scale diabetes register and biobank aiming at recruiting

and characterizing all new cases of diabetes in the county of Scania in southern Sweden by means of clinical and genetic information (15). To ESTRID we have invited all incident cases of LADA registered in ANDIS since 2010 together with a random sample of incident cases of type 2 diabetes. Non-diabetic control subjects within ESTRID provide questionnaire information but no laboratory data (n=1938). We included data for non-diabetic control subjects from the EIRA-study (Epidemiological Investigation of Rheumatoid Arthritis; https://www.eirasweden.se)(16), an ongoing case-control study based on similar methodology and questionnaire but also including biobank data. These control subjects are randomly selected from the Swedish population register and matched to the cases by age and gender. Analyses presented in this paper are based on all cases of LADA (n=394) and type 2 diabetes (n=1,290) participating in ESTRID between 2010 and July 2017 and controls (inclusion criteria were age ≥35 years and free of diabetes and rheumatoid arthritis, n=2,656), recruited in EIRA between 1996 and 2014, with information on BMI, all covariates and at least one of the genotypes of interest. All participants provided written informed consent and the study was approved by the Regional Ethical Review Board in Stockholm.

In ANDIS, blood samples were taken at diagnosis and diabetes classification was based on age at diagnosis, glutamic acid decarboxylase autoantibodies (GADA) and fasting C-peptide. Analyses of GADA were performed using ELISA (enzyme-linked immunosorbent assay; RSR Limited, Cardiff, UK). Values above 250 IU/ml are censored at 250 IU/ml and the assay has a sensitivity of 84% and specificity of 98% for a cut-off level of 10.7 IU/ml (17). C-peptide was determined using the Cobas e 601 analyzer (Roche Diagnostics, Mannheim, Germany) or IMMULITE 2000 (Siemens Healthcare Diagnostics Product Ldt., Llanberies, UK). LADA was defined as age at diabetes diagnosis ≥35 years, GADA positivity (≥10 IU/ml), and C-peptide level above the lower limit of the normal range; ≥0.2 nmol/l (IMMULITE) or ≥0.3 nmol/l (Cobas). The LADA definition is in line with previously used criteria (5) with the exception of C-peptide which has been suggested to be a more objective marker of remaining insulin production than the most commonly adopted insulin criterion i.e. free of insulin treatment 3-12 months after diagnosis (18). Criteria for type 2 diabetes were age ≥35 years, GADA negativity (<10 IU/ml) and C-peptide >0.6 nmol/l (IMMULITE) or >0.72 nmol/l (Cobas).

DNA samples from the ANDIS biobank were analyzed at the Lund University Diabetes Centre using iPLEX Gold technology (Sequenom Laboratories, San Diego, CA, USA). Imputation for missing genotypes was performed on a subset using Infinium CoreExome v1.1 (Illumina, San Diego, CA,USA), based on the Haplotype Reference Consortium (http://www.haplotype-reference-consortium.org/; version r1.1 2016) reference panel. DNA samples from the EIRA biobank were analyzed with the Illumina Global Screening array or an Infinium Illumina 300K immunochip custom array (Illumina, San Diego, CA, USA). For the present study we used SNPs in the HLA region (rs3104413, rs2854275, rs9273363), the *TCF7L2* gene (rs7903146) and the *FTO* gene (rs9939609). The HLA DRB1 and DQB1 genotypes associated with high (DR4/4, DR3/3, DR3/4, DR3/4-DQ8, DR4/4-DQ8, DR4/X-DQ8) or low/intermediate (DR4/X, DR3/X, DRX/X, DR4-DQ7) risk for type 1 diabetes were imputed from the SNP genotypes based on a previously described methodology validated with an overall accuracy of 99.3% (19).

Information on BMI and covariates was collected by questionnaire for all cases and controls at inclusion (median 5 months after diagnosis for cases). Self-reported BMI was calculated as weight in kilograms divided by the square of height in meters, which shows high correlation with BMI based on clinical measurements from time of diagnosis (patients only) (r=0.92, p <0,0001 [mean and standard deviation; 30.4 (5.5) for self-reports vs. 30.8 (5.6) for medical records, n=1622 (LADA n=376, type 2 diabetes n=1246)]). Detailed information on smoking history was used to categorize individuals as never, former or current

smokers. Physical activity was assessed with validated questions on leisure time activity (20) during the preceding year and categorized as sedentary, low, moderate or high activity.

The HUNT Study

The HUNT Study (21) consists of three health surveys (HUNT1, 1984-1986; HUNT2, 1995-97; HUNT3, 2006-08) conducted in the Norwegian county of Nord-Trøndelag. The surveys target all residents ≥ 20 years and include detailed questionnaires on lifestyle and health, clinical examinations, anthropometrical measurements and blood sampling. For the present study, the analytical sample comprised all individuals free of diabetes at baseline, who participated in at least two surveys with baseline information on BMI, age, gender, physical activity and smoking as well as genotypes for HLA, *TCF7L2* or *FTO* (n=48,599). Participants provided informed consent and the study was approved by the Norwegian Data Protection Authority and the Regional Committee for Medical and

Health Research Ethics.

Incident cases of diabetes were identified by self-report at HUNT2 or HUNT3 (correlation with diagnosis from medical records was high; r=0.92 (22). At screening all cases with self-reported diabetes were invited to a follow-up examination where fasting blood samples were collected (median 4 years after diagnosis). Serum C-peptide measurements (not from time of diagnosis) were analysed by RIA (Diagnostic Systems Laboratories, Webster, TX, USA). Serum GADA measurements were performed by immunoprecipitation radioligand assay using translation labelled ^{3}H-GAD65 (Novo Nordisk, Bagsværd, Denmark) and expressed as an index value relative to standard serum. In the IASP 2003 workshop the assay had a sensitivity of 0.64 and a specificity of 1.00 at the cut-off antibody index ≥0.08 used in the present study, which equals ≥43 IU/ml according to the WHO standard (23). LADA (n=131) was defined as age ≥35 years at diagnosis and GADA positivity, and type 2 diabetes (n=1,901) as age ≥35 years and GADA negativity. With these criteria, the LADA group will inevitably include also type 1 diabetes patients with adult onset. However, the proportion is likely to be small; among those with information on treatment (87.8%, n=105), the majority (81.7%) of the GADA positive patients with adult onset reported that they were without insulin treatment during the first year after diagnosis.

DNA was extracted for all participants in HUNT2 and HUNT3 with available blood samples and genotyped for SNPs associated with HLA-*DRB1* and HLA-*DQB1*, *TCF7L2* (rs7903146) and *FTO* (rs9939609) at the NTNU Genomic Core Facility, Trondheim by HumanCoreExome, Illumina Inc (San Francisco, CA, USA https://www.ntnu.no/hunt/gwas-data). Imputation was performed using Minimac3 (v2.0.1, http://genome.sph.umich.edu/wiki/Minimac3) and a customized Haplotype Reference consortium release 1.1 (HRC v1.1). Three SNPs, tagging the HLA region were available in HUNT (rs2854275, rs9273363, rs9272346) out of which two (rs2854275 and rs9273363) were the same as in ESTRID. Participants with at least one of the risk genotypes; rs2854275 (TT/TG), rs9273363 (AA) or rs9272346 (AA) were considered to have a HLA high-risk genotype. Selection of the included SNPs was based on publicly available results from LADA and type 1 diabetes studies (13,15,19) and the availability in our data.

Weight and height from the baseline clinical examination were used to calculate BMI as kg/m^2. Baseline questionnaire information was used to classify individuals into never, former or current smokers and to determine level of leisure time physical activity as sedentary, low, moderate or high activity.

In both cohorts, fasting C-peptide and glucose were used to calculate homeostasis model assessment indices (24) of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B).

Statistical analyses

Differences in baseline characteristics were assessed with two-sided p-values, calculated with Student's t-test for means (SD), Kruskal–Wallis for medians (interquartile range [IQR]) of non-normally distributed variables (normality was assessed by visual evaluation of the distribution), and $\chi 2$ for proportions. The association between genotype and LADA/type 2 diabetes was assessed by odds ratios (ORs) with 95% confidence intervals (CIs) derived by conditional logistic regression for case–control data and hazard ratios (HRs) with CIs calculated by proportional Cox regression for prospective data, modelled with age as the underlying time scale. For convenience, we will use the term relative risk (RR) to describe the effect estimates in present study. In HUNT, person-years were calculated from age at baseline until age at onset of diabetes, death or age at follow-up (in HUNT2 or HUNT3), whichever came first. Time-dependent variables were used, meaning that for individuals participating in both HUNT1 and HUNT2, information on exposure and covariates was updated at the second time of participation. All analyses were adjusted for age and gender (conditional variables in the case-control study) and where specified, additionally for physical activity and smoking. Additional adjustment for education (primary school, upper secondary school or university) and alcohol consumption (abstainers, low, moderate or high consumers) did not influence the effect estimates (<10% change) and these variables were not included in the final model. Study-specific RRs were calculated and pooled using the inverse variance method (25). Both *TCF7L2*-rs7903146 (TT/TC vs. CC) and *FTO*-rs9939609 (AA/AT vs. TT) were analyzed with a dominant model. In the specific HLA *DR-DQ* genotype analyses only Swedish data were used because we did not have SNPs tagging the DR4 genotype in HUNT. Patients with LADA were stratified by median GADA level (206 IU/ml [ESTRID] and 151 IU/ml [HUNT]), from here on referred to as $LADA^{high}$ and $LADA^{low}$. In HUNT, we also performed sensitivity analyses based on a stricter definition of LADA (i.e. no insulin treatment during the first year of diagnosis). Interaction between overweight and genotype, defined as departure from additivity of effects (super-additive associations), was assessed with attributable proportion (AP) due to interaction using the formula: $([RR_{11} - RR_{10} - RR_{01} + 1] / RR_{11})$ (26) where RR_{11} is the risk among those with overweight and the high-risk genotype, RR_{10} those with overweight and the low/intermediate risk genotype and RR_{01} those with normal weight and the high-risk genotype). The reference group was normal weight individuals without the genetic risk variant of interest. In an additional interaction analysis we raised the cutoff for BMI to ≥30 kg/m2 to assess the impact of the genotypes in obese vs. non-obese individuals. All statistical calculations were performed with Statistical Analysis Software (SAS) 9.4 (SAS Institute, Cary, NC, USA).

Results

Characteristics

In ESTRID (*Table 1*) individuals with LADA were leaner and younger, had lower insulin secretion (C-peptide and HOMA-B) but were less insulin resistant (HOMA-IR) than those with type 2 diabetes. The control subjects were younger and more often females than the patients, this difference was handled by post-matching in the subsequent analyses. In HUNT, patients with LADA and type 2 diabetes differed less than in ESTRID but the LADA patients had lower levels of C-peptide and they were more often treated with insulin.

Genotype and risk of LADA and Type 2 diabetes

As expected, HLA high-risk genotypes were associated with LADA in both cohorts, RR_{pooled} 2.74 (95% CI 2.23-3.36). *TCF7L2* was associated with LADA only in ESTRID (ESTRID *vs.* HUNT: RR 1.30 (95% CI 1.03-1.64) *vs.* 1.00 (95% CI 0.71-1.41); RR_{pooled} 1.20 (95% CI 0.99-1.45), while *FTO* was significantly associated with LADA only in HUNT (RR 1.11 (95% CI 0.86-1.42) *vs.* RR 1.65 (95% CI 1.10-2.46); RR_{pooled} 1.24 (95% CI 1.00-1.53) (*Figure 1*). Stratifying the analyses by median GADA level indicated that the association

with HLA was stronger for LADA^{high} than for LADA^{low} while the association with *TCF7L2* and *FTO* was restricted to LADA^{low} (*Supplemental figure 1*) (27). When restricting the analysis to LADA^{low}, the results regarding *TCF7L2* and *FTO* were more consistent across studies (*Supplemental figure 1*). Using a stricter LADA definition in HUNT, i.e. no insulin treatment, did not change the results (*Supplemental table 1*) (27). The highest risk of LADA was seen in those homozygous for the HLA DR4 allele (OR 13.73, 95% CI 9.35-20.18) out of whom 91% also had the DQ8 risk genotype (*Supplemental figure 2 and Supplemental table 2* [ESTRID]) (27). In both studies, type 2 diabetes was associated with *TCF7L2* and *FTO* but not with HLA (*Figure 1*).

Gene-overweight interaction and risk of LADA

Individuals with a combination of overweight (BMI ≥ 25 kg/m2) and HLA high-risk genotypes had almost 8-fold increased risk of LADA (RR_{pooled} 7.59, 95% CI 5.27-10.93) (*Table 2*) and a combination of DR4/4 and overweight appeared most detrimental (RR 26.76, 95% CI 15.42-46.43 [ESTRID]) (*Figure 2 and Supplemental table 3*) (27). In comparison, the combination of overweight with *TCF7L2* and *FTO* risk alleles yielded an RR_{pooled} of 2.65 (95% CI 1.97-3.56) and 2.21 (95% CI 1.60-3.07), respectively.

Moreover, there was significant interaction between overweight and all genotypes; AP_{pooled} was 0.29, 95% CI 0.10-0.47 (HLA); 0.31, 95% CI 0.09-0.52 (*TCF7L2*), and 0.38, 95% CI 0.15-0.61 (*FTO*) suggesting that around 29-38% of the LADA cases exposed to both risk factors might be prevented by maintaining a normal weight (*Table 2*). Overall, these results were consistent across cohorts (*Table 2*). The interaction between overweight and HLA was primarily observed for LADA^{high} and the interaction with *TCF7L2* primarily with LADA^{low} (*Supplemental table 4*) (27).

The combination of obesity (BMI ≥ 30 kg/m2) and risk genotypes yielded an even higher risk of diabetes (*Supplemental table 5*) (27), notably individuals with obesity and HLA risk genotypes had an RR_{pooled} of LADA 9.20 (6.53-12.96), with AP_{pooled} estimated at 0.41 (0.21-0.62).

Gene-overweight interaction and risk of type 2 diabetes

Interaction was observed between overweight and the *TCF7L2* risk allele; the joint exposure increased the risk ten-fold (RR_{pooled} 10.14, 95% CI 8.42-12.22) which corresponded to an AP_{pooled} of 0.26, 95% CI 0.19-0.33 (*Table 3*). No interaction was observed between overweight (*Table 3*) and either *FTO* or HLA. Findings were similar in obese vs. non obese individuals (*Supplemental table 5*) (27).

Discussion

We found a striking interaction between overweight/obesity and high-risk HLA genotypes in relation to the risk of LADA. Furthermore, the risk conferred by excess weight was especially high in those with the DR4/4 genotype. We also present novel results suggesting that excess weight interacts with *TCF7L2* and *FTO* risk alleles in the promotion of LADA but only with *TCF7L2* in promoting type 2 diabetes.

We confirm that HLA is strongly associated with the risk of LADA (6-8). The association with *TCF7L2* and *FTO* was much weaker and primarily seen for LADA with low GADA levels. Previous studies on LADA and *FTO* (12,13,28) and *TCF7L2* (9-13,29) have yielded inconsistent results, including a recent GWAS which did not find an association with either genotype (8). The lack of association may reflect limited power, especially in the GWAS where the nominal p-value was significant but not at the genome-wide significance level. It may also reflect the heterogeneity of LADA as shown here and in previous analyses of *TCF7L2* in relation with high and low GADA levels (11,12,29). With regard to type 2

diabetes, we confirm earlier studies indicating that the risk is associated with *TCF7L2* and *FTO* but not HLA (5).

The highest risk of LADA was seen in individuals with a combination of overweight/obesity and high-risk HLA genotypes. The HLA-complex is responsible for regulating the immune system and polymorphisms in this region are associated with development of insulin deficiency, presumably through autoimmune destruction of the pancreatic beta-cells (30). The influence of excess weight on LADA risk may be mediated by insulin resistance (4). One can hypothesize that when insulin resistance increases the need of insulin and the beta-cells fail to compensate for this need due to genetically determined insulin deficiency, the risk of diabetes escalates. This fits with the accelerator hypothesis which proposes that both type 2 diabetes and autoimmune diabetes are the result of disequilibrium between insulin sensitivity and insulin production (31). Another possibility is that high BMI promotes autoimmunity and adds to the genetically induced autoimmune reactivity. In support of that suggestion, Rolandsson *et al* found a positive association between BMI and GADA levels in a sample of non-diabetic individuals (32). Moreover, higher BMI has been associated with higher expression of the GAD2 gene in beta-cells of non-diabetic subjects (33). In apparent contrast to these observations, several studies have shown an inverse association between BMI and GADA levels among LADA patients (4,5,34); although one study showed that antibodies to a fragment of IA2 (protein tyrosine phosphatase islet antigen-2 $_{(256\text{-}760)}$) was associated with obesity in LADA patients (35). However, this inverse association between two risk factors of LADA may reflect collider bias due to restricting the study sample to patients with LADA (36).

The strongest interaction was observed in overweight individuals with the DR4/DR4 genotype, which to our knowledge has not been investigated previously. In this context it is noteworthy that significant interaction between HLA-DRB1*15 and adolescent obesity is observed in relation to the risk of multiple sclerosis (37). Further support is found in studies of type 1 diabetes in children; a Swedish case-only study (38) report a significant synergistic effect between overweight and HLA high-risk genotypes and results from the TEDDY study suggests that the effect of HLA-DQ2/2 genotype on diabetes risk could be mediated by obesity (39).

We also observed interaction between overweight and *TCF7L2* in relation to both LADA and type 2 diabetes. The *TCF7L2* locus is involved in the function of beta-cells and the rs7903146 risk variant is associated with increased gene expression in islets and reduced insulin secretion in a non-autoimmune fashion (40). Consequently, the excess risk seen in those exposed to both risk factors could be explained by failure of the beta-cells in carriers of *TCF7L2* to compensate for obesity induced insulin resistance in a similar way as described above. We did not find any previous reports of the combined effect of high BMI and *TCF7L2* on neither LADA nor type 2 diabetes, but if we recalculate the numbers presented in table 1 in the Hungarian LADA study by Lukacs *et al.* (10) there is no interaction and in contrast to our findings, no overall association between overweight and LADA is seen. These inconsistences may relate to differences in the populations or in study design, e.g. the Hungarian study was based on prevalent cases that were both younger and leaner than those included in this study. However, in keeping with our results are data from Cauchi *et al.* (41); when we recalculate the numbers in table 1 the results indicate additive interaction between obesity and the *TCF7L2* TT/TC genotype with AP estimated to 18.4%.

Similarly, a significant interaction between overweight and *FTO* was seen in LADA. The *FTO* gene is suggested to impact energy homeostasis control, with increased hunger and increased body fat in those with the risk variant (42). It has also been suggested that the impact of *FTO* is modulated by diet and eating behavior (42). A mechanistic explanation may be that environmental risk factors exaggerate the risk of overweight and subsequent insulin

resistance and diabetes in genetically susceptible individuals. Clearly these findings need confirmation, especially since we did not find any interaction for type 2 diabetes.

The strengths of this study include the possibility to replicate our findings in two large independent populations, the enrollment of incident cases as well as the detailed information on potential confounders. The self-reported information on weight and height in ESTRID is a drawback that may have led to an underestimation of the association between BMI and LADA/type 2 diabetes (43). We were able to validate the self-reports of the patients and found the correlation with information from their medical records to be high. In HUNT we had the advantage of using anthropometric measurements. In ESTRID, cases were matched to controls from another study population. The validity of this approach is supported by the fact that the results regarding LADA/type 2 diabetes and BMI was similar to those of a previous report where we used the controls recruited within ESTRID, for whom we did not have genetic information (4). Related to control sampling is also the fact that different assays were used for genotyping the cases and controls, thus a possible batch effect could have impacted these results. Such effect is most likely minor since our findings are in agreement with what has been reported previously (5,10-12). With regard to diagnostic criteria, LADA was distinguished from type 2 diabetes by means of only one autoantibody. This implies that some patients with autoimmune diabetes may have been included in the type 2 diabetes group. The proportion is likely to be small since GADA has been shown to be the most common autoantibody in LADA; 90% of LADA patients in the Action LADA study and 99% in a sub-study of HUNT were positive for GADA(44), (45). Also, some LADA patients may have converted to seronegative after diagnosis in the HUNT Study, in which GADA were assessed several years after diagnosis(45). Importantly, the lack of association between HLA risk genotypes and type 2 diabetes indicates that the proportion of misclassified autoimmune diabetes patients was small. In addition, the risk associated with *TCF7L2* was similar in those with low and high GADA levels in ESTRID and in a previous study from Finland[11], although not in HUNT. Moreover, even though specificity of the GADA assay was high, it is possible that some type 2 diabetes patients were misclassified as having LADA. Notably we observed a strong association with HLA high-risk genotypes and LADA, including LADA^{low}, which indicates that we did manage to isolate an autoimmune patient group. The criteria used to distinguish between LADA and type 1 diabetes with adult onset differed across studies; we used C-peptide levels in ESTRID and lack of insulin treatment within the first year following diagnosis in HUNT (sensitivity analysis). Although the LADA population in HUNT by definition is enriched by type 1 diabetes subjects, their proportion of GADA positive patients in that age-group is likely to be small, which is supported by the fact that they tended to have a more type 2-like phenotype than the LADA patients in ESTRID with higher HOMA-B levels, higher mean BMI and a stronger association with the *FTO* risk variant. This points at the heterogeneous nature of LADA (46). In this context it is important to note that results regarding interaction between BMI and HLA in relation to LADA risk were consistent across studies. Insulin treatment was more common in ESTRID than in HUNT but this most likely reflects differences in treatment strategies than in phenotype; although debated (46), early intervention with insulin has been suggested to preserve endogenous insulin secretion in LADA (18) and this could explain the frequent insulin treatment of the Swedish patients. With respect to generalizability, this report is based on Scandinavia populations; hence replications in other populations with different ethnic and genetic background are warranted in order to generalize the findings.

Conclusions

In conclusion, our study suggests that overweight strongly interacts with HLA high-risk genotypes but also with risk variants of *TCF7L2* and *FTO* in LADA, indicating that excessive

weight is a particularly strong risk factor for LADA in individuals with genetic susceptibility. As such, our data put forward that lifestyle modification to maintain a healthy weight may be particularly important for prevention of LADA among people with HLA risk alleles.

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All authors contributed to the interpretation of the results and critically revised and approved the final version of the manuscript. Contributions to the data collection was made by; SC, RH, JEL and TA (ESTRID), LA (EIRA), LG, EA, AR, TT (ANDIS), EPS, VG and BOÅ (HUNT). JEL contributed to the data analysis. TA contributed with statistical expertise. SC was responsible for the conceptualized research objectives and designed the study and thoroughly revised the manuscript. RH developed the objectives of the study and was responsible for drafting of the manuscript and analyzing the data and takes full accountability for the accuracy of the analyses and the work as a whole.

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The authors have nothing to disclose.

Data Availability

Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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Figure 1. Relative risks with 95% confidence intervals of LADA and type 2 diabetes by genotypes of HLA (high-risk vs. low/intermediate risk), *TCF7L2* (TT/TC vs. CC), and *FTO* (AA/AT vs. TT).

Figure 2. Relative risks with 95% confidence intervals of LADA in relation to different combinations of BMI and genotypes of HLA DRB1 in the ESTRID study. *Reference is normal weight individuals with low/intermediate risk HLA genotypes. Adjusted for age and gender.

Table 1. Characteristics of study participants

	ESTRID				**HUNT**			
	Controls	LADA	Type 2 diabetes	*p*[a]	No diabetes	LADA	Type 2 diabetes	*p*[a]
No. of individuals	2,656	394	1,290		46,567	131	1,901	
Men, %	27.6	53.0	60.1	*0.0132*	46.7	48.9	52.8	*0.3864*
Age at diagnosis[b], years, mean (SD)	56.6 (9.4)	58.6 (12.3)	63.2 (10.3)	*<0.0001*	–	59.5 (11.2)	60.7 (10.9)	*0.1914*

	ESTRID				HUNT			
	Controls	LADA	Type 2 diabetes	p[a]	No diabetes	LADA	Type 2 diabetes	p[a]
Age, years, at baseline (HUNT), mean (SD)	–	–	–	–	48.9 (15.8)	54.0 (11.4)	54.6 (11.0)	*0.5418*
BMI (kg/m^2), mean (SD)	25.5 (4.1)	27.9 (5.2)	31.2 (5.3)	*<0.0001*	25.9 (3.8)	29.1 (4.8)	29.8 (4.5)	*0.1066*
Overweight, %	47.5	70.1	93.4	*<0.0001*	56.2	82.4	88.3	*0.0456*
With insulin treatment, %	–	47.4	5.8	*<0.0001*	–	45.8	15.0	*<0.0001*
GADA (IU/ml), median (IQR)	–	206 (27-250)	–	–	–	151 (59-613)	–	–
C-peptide (nmol/l), median (IQR)[c]	–	0.66 (0.42-1.10)	1.20 (0.95-1.60)	*<0.0001*	–	0.53 (0.18-0.91)	0.86 (0.60-1.20)	*<0.0001*
HOMA-B, median (IQR)[c]	–	34.2 (13.3-65.1)	68.1 (42.5-92.8)	*<0.0001*	–	58.5 (36.6-81.9)	65.3 (43.8-92.9)	*0.2696*
HOMA-IR, median (IQR)[c]	–	2.70 (1.80-4.40)	3.60 (2.70-4.80)	*<0.0001*	–	2.05 (1.10-2.70)	2.20 (1.60-3.10)	*0.0547*
High-risk HLA, %	33.0	61.2	31.4	*<0.0001*	42.5	60.3	40.7	*<0.0001*
TCF7L2-rs7903146, %[d]	45.8	51.8	52.1	*0.7795*	44.2	44.3	54.9	*0.0186*
FTO-rs9939609, %[e]	64.33	66.3	67.5	*0.5156*	65.9	76.3	69.2	*0.0847*

a) P for difference between LADA and type 2 diabetes. b) Age at inclusion for control participants in ESTRID. c) Clinical information was available for 100% of the patients in ESTRID and 70% of patients in HUNT (LADA n=105, type 2 diabetes n=1,316). d) Proportion with the TT/TC genotype. e) Proportion with the AA/AT genotype. HLA high-risk: DR4/4, DR3/3, DR3/4, DR3/4-DQ8, DR4/4-DQ8 or DR4/X-DQ8.

Table 2. Relative risks (RR) and attributable proportion due to interaction (AP) with 95% Confidence Intervals (CI) in relation to LADA for different combinations of BMI and genotypes of HLA, *TCF7L2* and *FTO*.

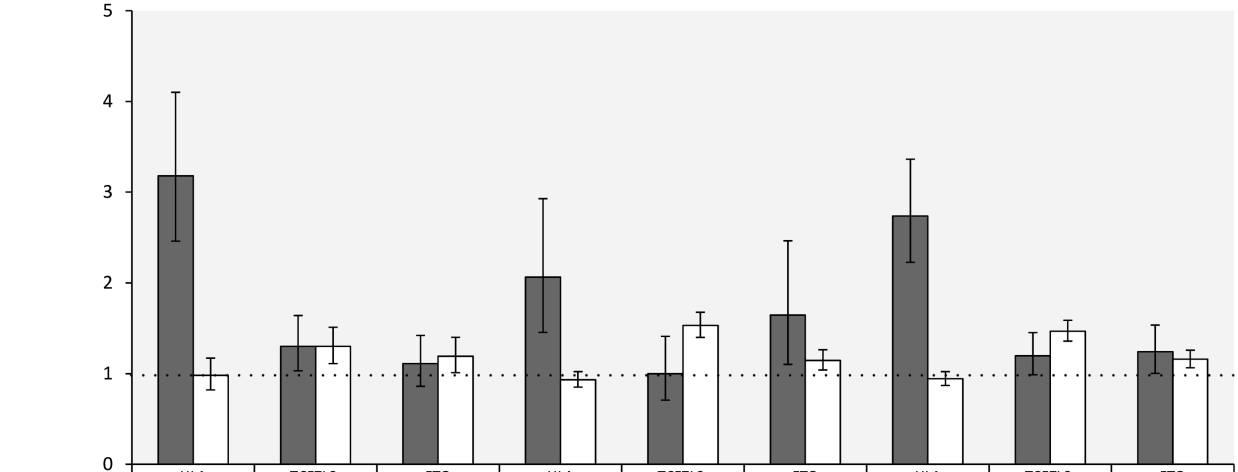
		ESTRID			HUNT			Pooled
		No. cases	No. controls	RR (95% CI)	No. cases	Person-years	RR (95% CI)	RR (95% CI)
BMI ≥25	**HLA** high-risk							
-	-	42	637	1 (Reference)	8	251,825	1 (Reference)	1 (Reference)
+	-	111	587	2.57 (1.69-3.92)	44	258,428	3.86 (1.81-8.26)	2.83 (1.96-4.09)
-	+	76	325	3.84 (2.43-6.05)	15	186,239	2.55 (1.08-6.01)	3.51 (2.35-5.25)
+	+	165	277	7.56 (4.97-11.50)	64	189,628	7.67 (3.65-16.09)	7.59 (5.27-10.93)
	AP (95% CI)			*0.28 (0.05-0.52)*			*0.29 (0.00-0.59)*	*0.29 (0.10-0.47)*
BMI ≥25	***TCF7L2*** TT/TC							
-	-	58	756	1 (Reference)	18	241,094	1 (Reference)	1 (Reference)
+	-	121	672	1.91 (1.34-2.73)	55	251,403	2.10 (1.22-3.61)	1.97 (1.46-2.65)
-	+	55	631	1.19 (0.79-1.80)	5	196,969	0.34 (0.13-0.91)	0.43 (0.36-0.51)
+	+	137	576	2.67 (1.88-3.79)	53	196,654	2.60 (1.51-4.47)	2.65 (1.97-3.56)
	AP (95% CI)			*0.21 (-0.07-0.49)*			*0.45 (0.11-0.78)*	*0.31 (0.09-0.52)*
BMI ≥25	***FTO*** AA/AT							
-	-	47	475	1 (Reference)	9	156,631	1 (Reference)	1 (Reference)
+	-	78	387	1.61 (1.06-2.44)	22	144,128	1.91 (0.87-4.16)	1.67 (1.16-2.42)
-	+	66	794	0.80 (0.53-1.22)	14	281,432	0.87 (0.38-2.02)	0.81 (0.56-1.18)
+	+	180	761	1.93 (1.33-2.79)	86	303,929	3.58 (1.79-7.15)	2.21 (1.60-3.07)
	AP (95% CI)			*0.27 (-0.06-0.59)*			*0.50 (0.17-0.83)*	*0.38 (0.15-0.61)*

Adjusted for age, gender, smoking and physical activity. HLA high-risk: DR4/4, DR3/3, DR3/4, DR3/4-DQ8, DR4/4-DQ8 or DR4/X-DQ8.

Table 3. Relative risks (RR) and attributable proportion due to interaction (AP) with 95% confidence intervals (CI) in relation to type 2 diabetes for different combinations of BMI and genotypes of HLA, TCF7L2 and FTO.

		ESTRID			**HUNT**			**Pooled**
		No. cases	No. controls	RR (95% CI)	No. cases	Person-years	RR (95% CI)	RR (95% CI)
BMI ≥25	**HLA** high-risk							
-	-	57	637	1 (Reference)	124	251,825	1 (Reference)	1 (Reference)
+	-	825	587	16.49 (11.42-23.81)	1004	258,428	5.61 (4.65-6.77)	7.01 (5.93-8.29)
-	+	27	325	1.48 (0.85-2.58)	98	186,239	1.06 (0.82-1.39)	1.13 (0.89-1.43)
+	+	376	277	15.51 (10.50-22.90)	675	189,628	5.16 (4.25-6.26)	6.42 (5.39-7.63)
	AP (95% CI)			*-0.09 (-0.34-0.15)*			*-0.10 (-0.22-0.02)*	*-0.10 (-0.21-0.01)*
BMI ≥25	**TCF7L2** TT/TC							
-	-	40	756	1 (Reference)	95	241,094	1 (Reference)	1 (Reference)
+	-	556	672	14.73 (10.01-21.68)	763	251,403	5.46 (4.40-6.77)	6.91 (5.72-8.34)
-	+	43	631	1.64 (1.00-2.70)	127	196,969	1.63 (1.25-2.12)	1.63 (1.29-2.06)
+	+	606	576	18.78 (12.74-27.69)	916	196,654	8.43 (6.82-10.43)	10.14 (8.42-12.22)
	AP (95% CI)			*0.18 (0.03-0.33)*			*0.28 (0.20-0.36)*	*0.26 (0.19-0.33)*
BMI ≥25	**FTO** AA/AT							
-	-	30	475	1 (Reference)	76	156,631	1 (Reference)	1 (Reference)
+	-	375	387	12.52 (8.01-19.58)	510	144,128	5.23 (4.10-6.66)	6.38 (5.15-7.90)
-	+	53	794	1.07 (0.64-1.79)	146	281,432	1.08 (0.82-1.43)	1.08 (0.84-1.38)
+	+	787	761	14.23 (9.24-21.92)	1169	303,929	5.72 (4.53-7.22)	7.13 (5.77-8.82)
	AP (95% CI)			*0.12 (-0.06-0.29)*			*0.07 (-0.04-0.18)*	*0.08 (-0.01-0.18)*

Adjusted for age, gender, smoking and physical activity. HLA high-risk: DR4/4, DR3/3, DR3/4, DR3/4-DQ8, DR4/4-DQ8 or DR4/X-DQ8.



	HLA	TCF7L2	FTO	HLA	TCF7L2	FTO	HLA	TCF7L2	FTO
LADA	3.18	1.30	1.11	2.06	1.00	1.65	2.74	1.20	1.24
Type 2 diabetes	0.98	1.30	1.19	0.93	1.53	1.15	0.94	1.47	1.16
	ESTRID			HUNT			Pooled